DENGUE LOCAL EPIDEMIOLOGY & DIAGNOSTIC APPROACH

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OUTLINE

- Introduction
- Transmission
- Clinical Manifestation
- Diagnostic Test
- Management
- Local Epidemiology

OVERLAPPING TERMINOLOGIES

Arboviruses

Short for **ARthropod-BOrne VIRUSES**.

Transmitted by vectors like **mosquitoes**, **ticks**, and **sandflies**.

Flaviviridae is one of several families that include arboviruses:

- **Flaviviridae**: Dengue, yellow fever, Zika
- **Bunyaviridae**: Rift Valley fever, Crimean-Congo hemorrhagic fever
- **Togaviridae**: Chikungunya
- **Reoviridae**: Colorado tick fever

OVERLAPPING TERMINOLOGIES

Viral Hemorrhagic Fevers (VHFs)

A clinical syndrome caused by viruses from multiple families, including:

- **Flaviviridae** (e.g., dengue, yellow fever)
- **Filoviridae** (e.g., Ebola, Marburg)
- **Arenaviridae** (e.g., Lassa fever)
- **Bunyaviridae** (e.g., CCHF, RVF)

Characterized by **fever, bleeding, and vascular damage**.

Many VHFs are **arboviral**, but not all (e.g., Lassa fever is rodent-borne).

INTRODUCTION: FLAVIVIRIDAE

Flaviviridae Family – Key Points

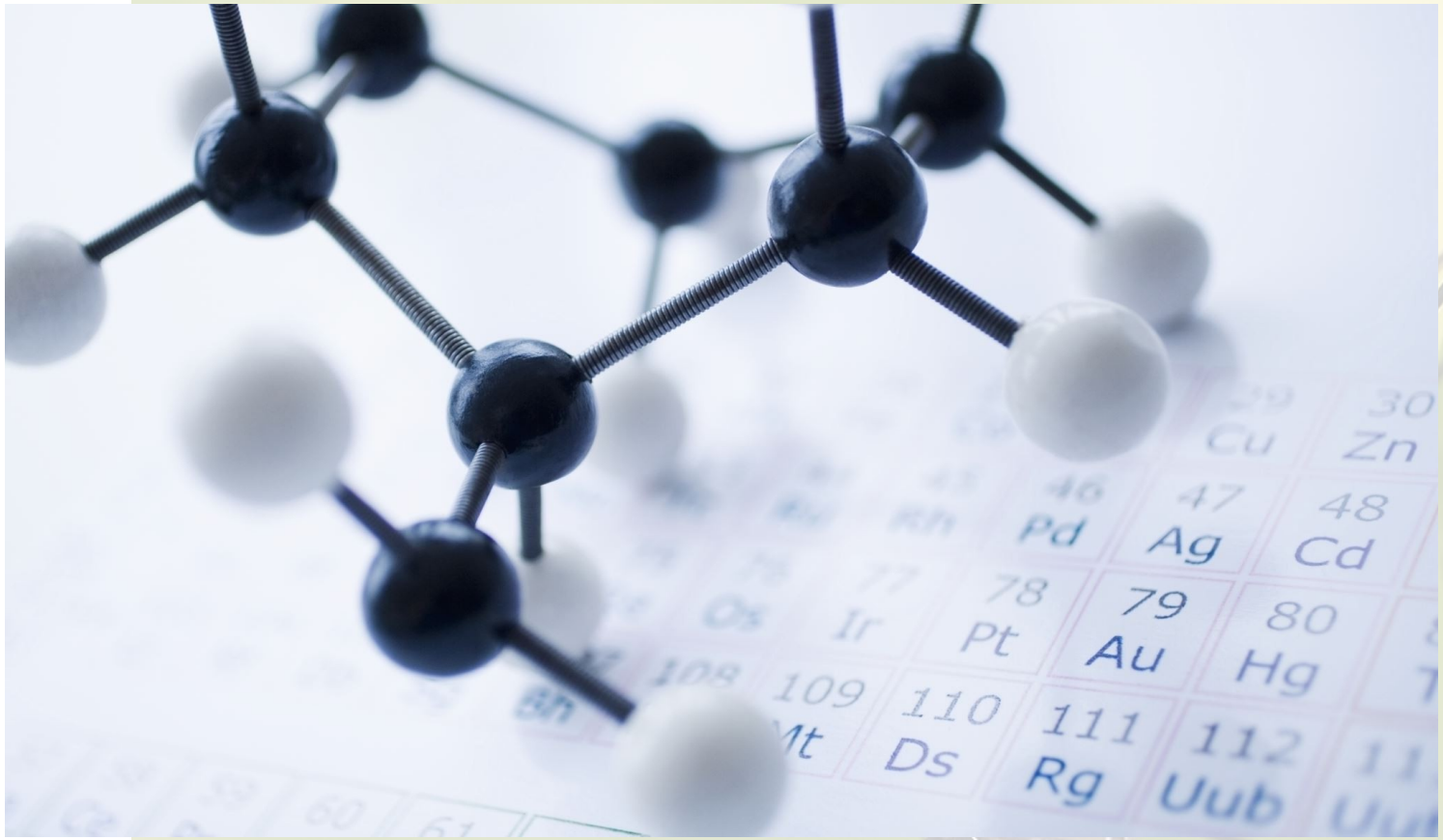
RNA viruses: Positive-sense, single-stranded, enveloped RNA genomes (10–12.5 kb)

Genera:

- *Flavivirus* (e.g., Yellow fever virus, Dengue, Zika) => Orthoflaviviruses
- *Hepacivirus* (e.g., Hepatitis C virus)
- *Pegivirus* (e.g., Human Pegivirus 1 aka: hepatitis G virus)
- *Pestivirus* (e.g., Bovine Viral Diarrhea Virus)

Genome features:

- Flaviviruses: 5′ methylated cap for translation
- Hepacivirus & Pestivirus: uncapped, use internal ribosome entry site (IRES)



INTRODUCTION: FLAVIVIRIDAE

- **Virion structure**:
- Enveloped, icosahedral/spherical
- Diameter: ~40–60 nm
- **Shared traits**:
- Similar virion morphology and genome organization
- Conserved replication patterns despite biological differences
- **Global impact**:
- Significant morbidity and mortality worldwide

INTRODUCTION: FLAVIVIRIDAE

- **Vector-borne transmission**:
- **Mosquito-borne**: Dengue, Yellow fever, Zika, West Nile, Japanese encephalitis
- **Tick-borne**: Kyasanur Forest disease (KFDV),
- Alkhurma hemorrhagic fever (AHFV), Omsk hemorrhagic fever (OHFV)
- **Hemorrhagic fever viruses in this family**:
- AHFV, KFDV, OHFV, YFV, DENV

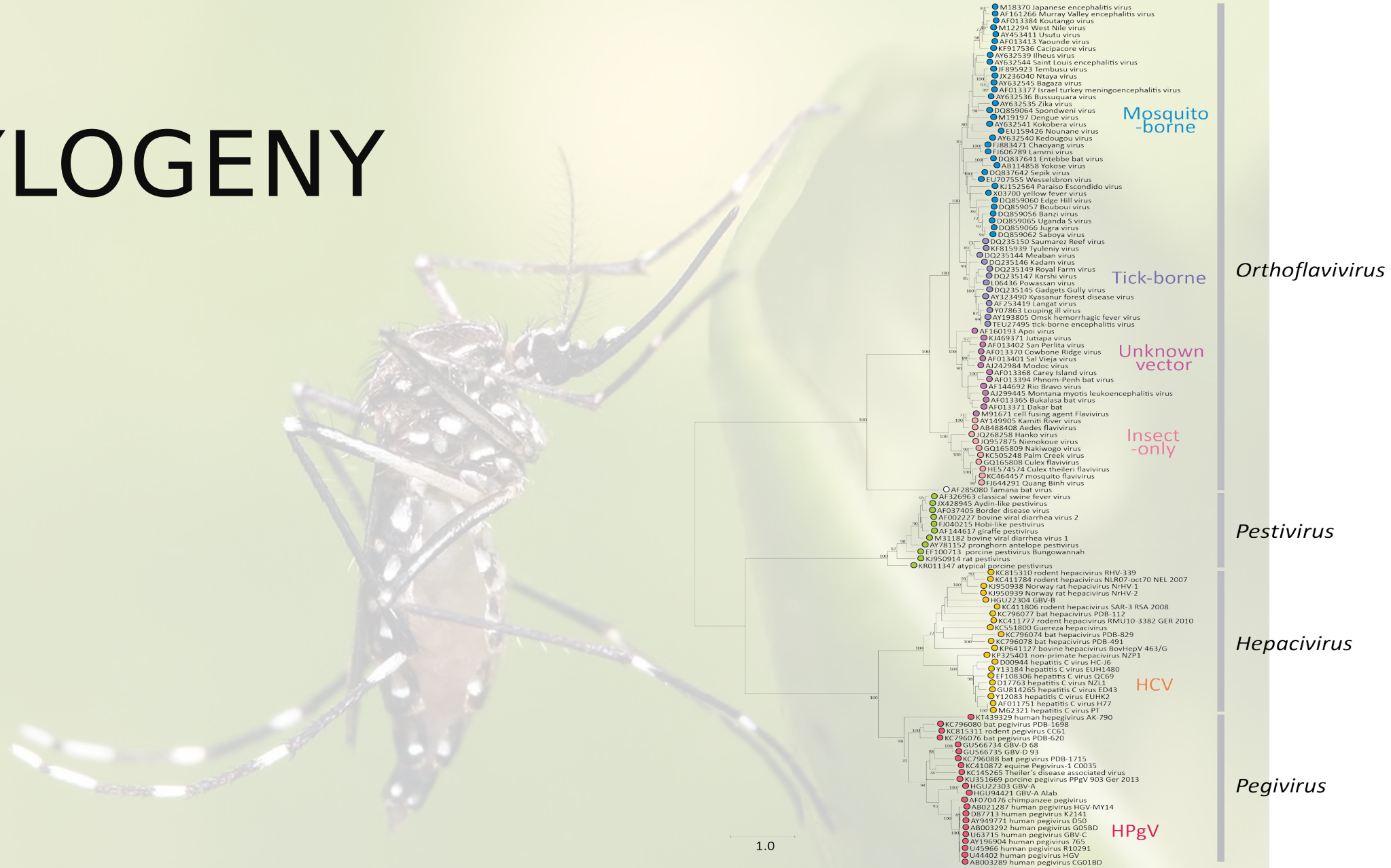
SUMMARY OF MAJOR ORTHOFLAVIVIRUSES

Table 121.4. Flaviviruses causing human hemorrhagic fever.

Virus	Vector	Principal reservoir	Distribution
AHFV	Sand tampan (*Ornithodoros savignyi*)	Camel, sheep	Saudi Arabia
KFDV	Ixodid ticks (*Haemaphysalis spinigera*)	Porcupines, rats, squirrels, mice, and shrews	India, Saudi Arabia
OHFV	Ixodid ticks (*Dermacentor sp.*)	Migratory birds, rodents	Russia
YFV	Mosquitoes (*Aedes aegypti*)	Nonhuman primates	Africa, South America
Dengue fever virus (DENV)	Mosquitoes (*A. aegypti*)	Humans, nonhuman primates	throughout the Tropics

AHFV, AHF virus; *DENV*, Dengue fever virus; *KFDV*, KFD virus; *OHFV*, OHF virus; *YFV*, Yellow fever virus.

PHYLOGENY


M18370 Japanese encephalitis virus
AF161266 Murray Valley encephalitis virus
AF013384 Koutango virus
M12294 West Nile virus
AY453411 Usutu virus
AF013413 Yaounde virus
KF917536 Cacipacore virus
AY632539 Ilheus virus
AY632544 Saint Louis encephalitis virus
JF895923 Tembusu virus
JX236040 Ntaya virus
AY632545 Bagaza virus
AF013377 Israel turkey meningoencephalitis virus
AY632536 Bussuquara virus
AY632535 Zika virus
DQ859064 Spondweni virus
M19197 Dengue virus
AY632541 Kokobera virus
EU159426 Nounane virus
AY632540 Kedougou virus
FJ883471 Chaoyang virus
FJ606789 Lammi virus
DQ837641 Entebbe bat virus
AB114858 Yokose virus
DQ837642 Sepik virus
EU707555 Wesselsbron virus
K152564 Paraiso Escondido virus
X03700 yellow fever virus
DQ859060 Edge Hill virus
DQ859057 Bouboui virus
DQ859056 Banzi virus
DQ859065 Uganda S virus
DQ859066 Jugra virus
DQ859062 Saboya virus
DQ235150 Saumarez Reef virus
KF815939 Tyuleniy virus
DQ235144 Meaban virus
DQ235146 Kadam virus
DQ235149 Royal Farm virus
DQ235147 Karshi virus
I06436 Powassan virus
DQ235145 Gadgets Gully virus
AY323490 Kyasanur forest disease virus
AF253419 Langat virus
Y07863 Louping ill virus
AY193805 Omsk hemorrhagic fever virus
TEU27495 tick-borne encephalitis virus
AF160193 Apoi virus
KJ469371 Jutiapa virus
AF013402 San Perlita virus
AF013370 Cowbone Ridge virus
AF013401 Sal Vieja virus
AJ242984 Modoc virus
AF013368 Carey Island virus
AF013394 Phnom-Penh bat virus
AF144692 Rio Bravo virus
AJ299445 Montana myotis leukoencephalitis virus
AF013365 Bukalasa bat virus
AF013371 Dakar bat
M91671 cell fusing agent Flavivirus
AY149905 Kamiti River virus
AB488408 Aedes flavivirus
JQ268258 Hanko virus
JQ957875 Nienokoue virus
GQ165809 Nakiwogo virus
KC505248 Palm Creek virus
GQ165808 Culex flavivirus
HE574574 Culex theileri flavivirus
KC464457 mosquito flavivirus
FJ644291 Quang Binh virus
AF285080 Tamana bat virus
AF326963 classical swine fever virus
JX428945 Aydin-like pestivirus
AF037405 Border disease virus
AF002227 bovine viral diarrhea virus 2
FJ040215 Hobi-like pestivirus
AF144617 giraffe pestivirus
M31182 bovine viral diarrhea virus 1
AY781152 pronghorn antelope pestivirus
EF100713 porcine pestivirus Bungowannah
KJ950914 rat pestivirus
KR011347 atypical porcine pestivirus
KC815310 rodent hepacivirus RHV-339
KC411784 rodent hepacivirus NLR07-oct70 NEL 2007
KJ950938 Norway rat hepacivirus NrHV-1
KJ950939 Norway rat hepacivirus NrHV-2
HGU22304 GBV-B
KC411806 rodent hepacivirus SAR-3 RSA 2008
KC796077 bat hepacivirus PDB-112
KC411777 rodent hepacivirus RMU10-3382 GER 2010
KC551800 Guereza hepacivirus
KC796074 bat hepacivirus PDB-829
KC796078 bat hepacivirus PDB-491
KP641127 bovine hepacivirus BovHepV 463/G
KP325401 non-primate hepacivirus NZP1
D00944 hepatitis C virus HC-J6
Y13184 hepatitis C virus EUH1480
EF108306 hepatitis C virus QC69
D17763 hepatitis C virus NZL1
GU814265 hepatitis C virus ED43
Y12083 hepatitis C virus EUHK2
AF011751 hepatitis C virus H77
M62321 hepatitis C virus P1
KT439329 human hepegivirus AK-790
KC796080 bat pegivirus PDB-1698
KC815311 rodent pegivirus CC61
KC796076 bat pegivirus PDB-620
GU566734 GBV-D 68
GU566735 GBV-D 93
KC796088 bat pegivirus PDB-1715
KC410872 equine Pegivirus-1 C0035
KC145265 Theiler's disease associated virus
KU351669 porcine pegivirus PPgV 903 Ger 2013
HGU22303 GBV-A
HGU94421 GBV-A Alab
AF070476 chimpanzee pegivirus
AB021287 human pegivirus HGV-MY14
DB7713 human pegivirus K2141
AY949771 human pegivirus D50
AB003292 human pegivirus G05BD
U63715 human pegivirus GBV-C
AY196904 human pegivirus 765
U45966 human pegivirus R10291
U44402 human pegivirus HGV
AB003289 human pegivirus CG01BD
1.0
Mosquito -borne
Tick-borne
Unknown vector
Insect -only
HCV
HPgV
Orthoflavivirus
Pestivirus
Hepacivirus
Pegivirus

INTRODUCTION

Dengue (break-bone fever) is a viral infection transmitted to humans through the bite of infected mosquitoes.

Dengue is caused by infection with one of the dengue viruses (Orthoflavivirus dengue, serotypes 1-4), belonging to the family Flaviviridae

About half of the world's population is now at risk of dengue with an estimated 100–400 million infections occurring each year (WHO)

TRANSMISSION

Dengue viruses are spread to people through the bites of infected *Aedes* species mosquitoes (*Ae. aegypti* or *Ae. albopictus*). These are the same types of mosquitoes that spread Zika and chikungunya viruses.

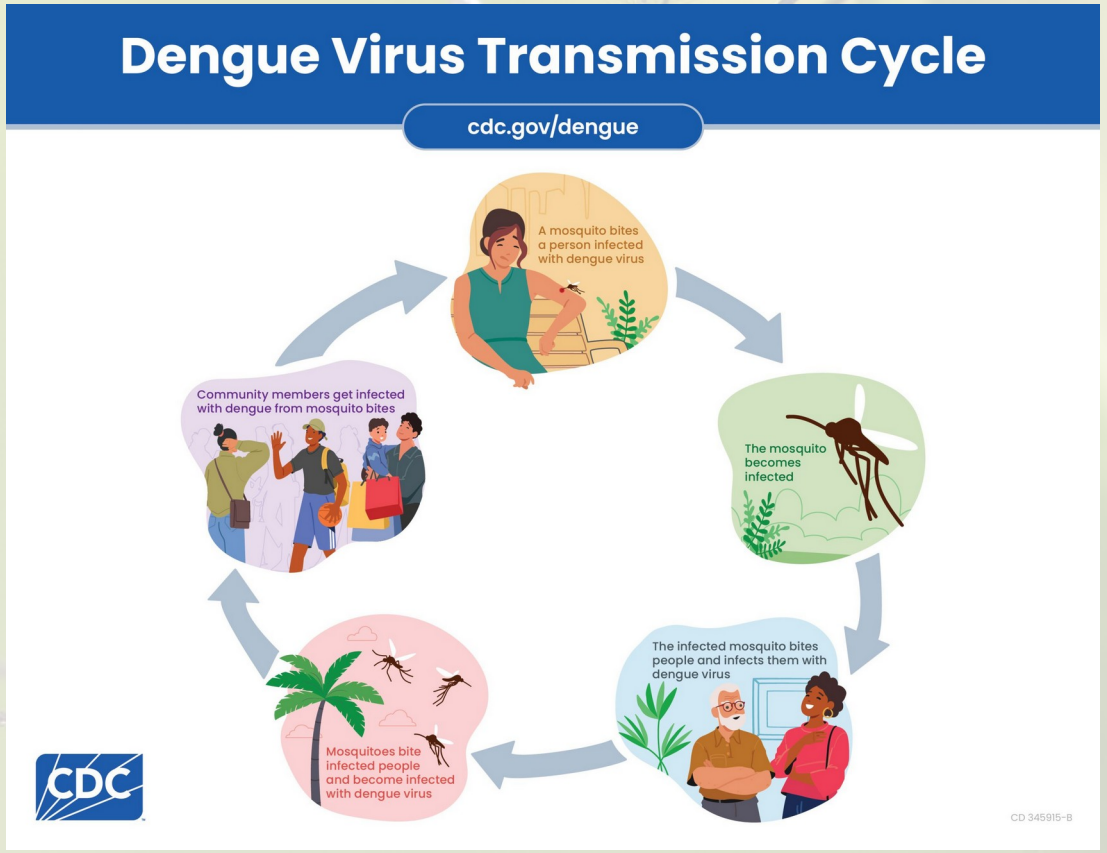
These mosquitoes typically lay eggs in containers that hold water, like buckets, bowls, animal dishes, flowerpots, and vases.

These mosquitoes prefer to bite people, and live both indoors and outdoors near people.

Aedes species mosquitoes can bite during the day and night.

Mosquitoes become infected with dengue virus when they bite a person infected with the virus. Infected mosquitoes can then spread the virus to other people through bites.

MOST COMMON WAY OF TRANSMISSION


Dengue Virus Transmission Cycle
cdc.gov/dengue
A mosquito bites a person infected with dengue virus
The mosquito becomes infected
The infected mosquito bites people and infects them with dengue virus
Mosquitoes bite infected people and become infected with dengue virus
Community members get infected with dengue from mosquito bites
CDC
CD 345915-B

TRANSMISSION

- The mosquito is not the main cause of the disease, but only a vector that carries the virus from one person to another.
- The incubation period ranges from 8 to 12 days, during which the virus multiplies in the mosquito's gut and settles in its salivary glands. The mosquito then start transmitting the disease for the rest of its life.
- Early morning and before sunset are two peak hours for mosquito bites.

PREGNANCY

A woman who is infected with dengue virus during pregnancy can pass the virus to her fetus during pregnancy or around the time of birth.

Dengue can have harmful effects, including death of the fetus, low birth weight, and premature birth.

THROUGH OTHER ROUTES

Rarely, dengue is spread in laboratory or healthcare setting exposures through blood transfusions, organ transplants, or needlestick injuries.

Some evidence suggests that dengue can be spread through sexual contact.

Dengue virus has been found in breast milk but transmission through breast milk has not been confirmed. Because of the low risk of transmission and the benefits of breastfeeding, women are encouraged to breastfeed even if dengue infection is suspected or confirmed.

DISTRIBUTION

- Almost half of the world's population, about 4 billion people, live in areas with a risk of dengue.
- Dengue outbreaks are reported frequently in these regions, including many popular tourist destinations in:

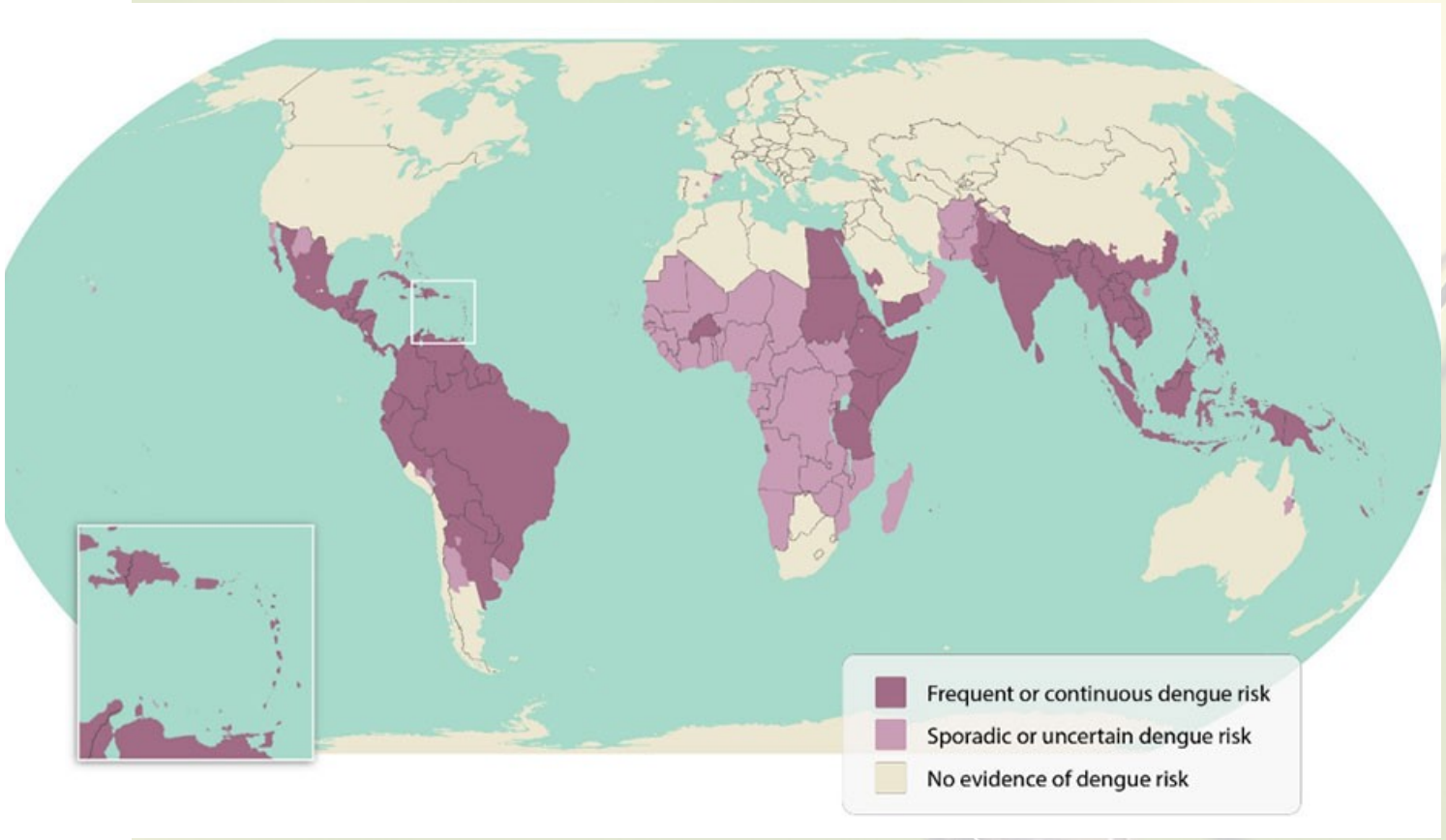
the Caribbean

Central America

South America

Southeast Asia

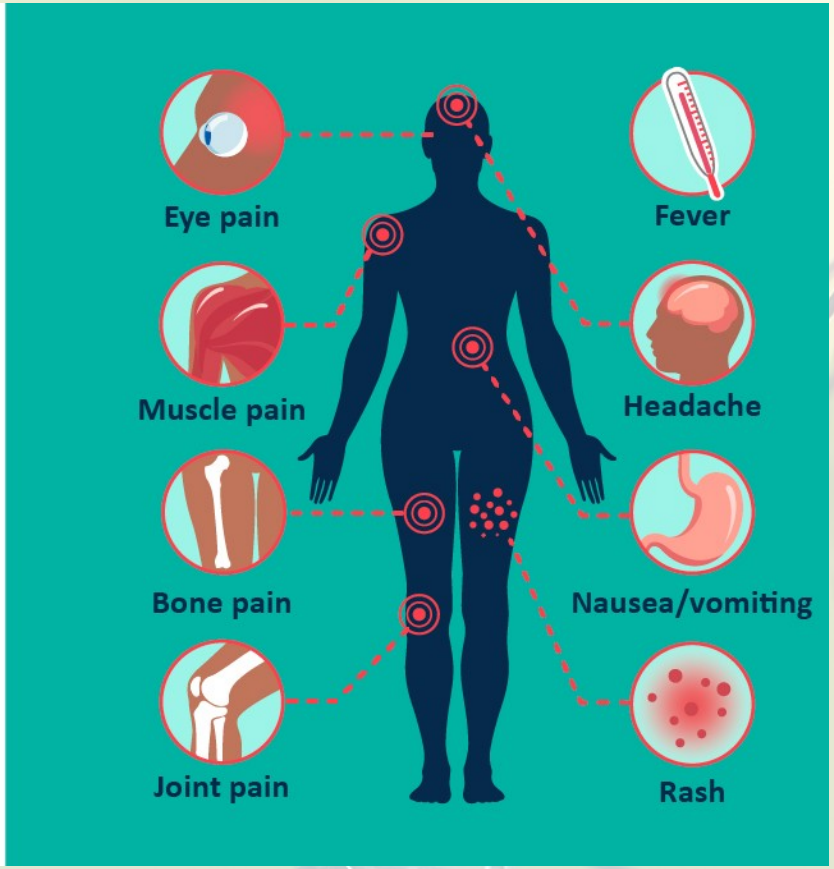
Pacific Islands



DISTRIBUTION

Dengue is considered endemic in western coastal areas of Saudi Arabia, including Jeddah and the Makkah region

CLINICAL MANIFESTATIONS



Symptoms begin to manifest 4-10 days after being bitten by an infective mosquito

- Febrile, critical and recovery phases

1- FEBRILE PHASE:

- Sudden high grade fever
- This acute febrile phase usually lasts 2−7 days
- Often accompanied by facial flushing, skin erythema, generalized body ache, myalgia, arthralgia, retro-orbital eye pain, photophobia, rubeliform exanthema and headache.
- Some patients may have a sore throat, an injected pharynx, and conjunctival injection. Anorexia, nausea and vomiting are common.
- Difficult to distinguish from non-dengue febrile diseases in this phase. A positive tourniquet test in this phase indicates an increased probability of dengue

1- FEBRILE PHASE:

- Mild hemorrhagic manifestations such as petechiae and mucosal membrane bleeding (e.g. of the nose and gums) may be seen . Easy bruising and bleeding at venipuncture sites is present in some cases.
- Massive vaginal bleeding and GI bleeding may occur during this phase although this is not common.
- The liver may be enlarged and tender after a few days of fever.
- The earliest abnormality in the full blood count is a progressive decrease in total white cell count, suggesting high probability of dengue.

DENGUE RASH

a confluent, non-blanching erythematous rash with macules and patches of normal skin, referred to as "white islands in a sea of red."

DENGUE RASH

This type of rash usually appears during defervescence and gradually fades over the course of a week

DENGUE RASH

Various factors are believed to contribute to the development of this rash, including viral replication in immune cells like Langerhans cells in the skin, direct skin infection by the dengue virus, and complex interactions between the virus and the host's immune response.

The characteristic "white islands in a sea of red" rash in dengue is a useful diagnostic clue, indicating increased capillary permeability and fluid leakage. This distinctive rash is a hallmark of dengue defervescence and aids in distinguishing dengue from other febrile illnesses with similar presentations.

2- CRITICAL PHASE

- During the transition from the febrile to afebrile phase, patients without an increase in capillary permeability will improve without going through the critical phase, while patients with increased capillary permeability may manifest with the warning signs, mostly as a result of plasma leakage.
- The warning signs mark the beginning of the critical phase, usually on days 3–8 of illness, which is around the time of defervescence.
- Progressive leukopenia followed by a rapid decrease in platelet count usually precedes plasma leakage.
- An increasing hematocrit above the baseline may be one of the earliest additional signs. The period of clinically significant plasma leakage usually lasts 24−48 hours. The degree of plasma leakage varies. A rising hematocrit precedes changes in blood pressure (BP) and pulse volume.
- This may be reduced by early IV fluid therapy. Hence, frequent hematocrit determinations are essential because they signal the need for possible adjustments to intravenous fluid therapy

WARNING SIGNS

- Usually precede the manifestations of shock and appear towards the end of the febrile phase, usually between days 3–7 of illness.
- Persistent vomiting and severe abdominal pain are early indications of plasma leakage and become increasingly worse as the patient progresses to the shock state. The patient becomes increasingly lethargic but usually remains mentally alert.
- These symptoms may persist into the shock stage. Weakness, dizziness or postural hypotension occur during the shock state. Spontaneous mucosal bleeding or bleeding at previous venipuncture sites are important hemorrhagic manifestations. Increasing liver size and a tender liver is frequently observed. However, clinical fluid accumulation may only be detected if plasma loss is significant or after treatment with intravenous fluids. A rapid and progressive decrease in platelet count to about 100 000 cells/mm3 and a rising hematocrit above the baseline may be the earliest sign of plasma leakage. This is usually preceded by leukopenia (≤ 5000 cells/mm

CRITICAL PHASE

Pleural effusion and ascites are usually only clinically detectable after intravenous fluid therapy. In addition to the plasma leakage, hemorrhagic manifestations such as easy bruising and bleeding at venipuncture sites occur frequently.

Hypovolemic Shock => hypoperfusion results in metabolic acidosis, progressive organ impairment, and disseminated intravascular coagulation. This in turn can lead to severe hemorrhage causing the hematocrit to decrease in severe shock. Instead of the leukopenia usually seen during this phase of dengue, the total white cell count may increase as a stress response in patients with severe bleeding. In addition, severe organ involvement may develop such as severe hepatitis, encephalitis, myocarditis, and/or severe bleeding, without obvious plasma leakage or shock

DENGUE SHOCK SYNDROME (DSS)

Dengue shock syndrome (DSS) is a form of hypovolaemic shock and results from continued vascular permeability and plasma leakage. This usually takes place around defervescence, i.e. on days 4−5 of illness (range of days 3−8), and is often preceded by warning signs. From this point onwards, patients who do not receive prompt intravenous fluid therapy progress rapidly to a state of shock. Dengue shock presents as a physiologic continuum, progressing from asymptomatic capillary leakage to compensated shock to hypotensive shock and ultimately to cardiac arrest

3-RECOVERY PHASE

As the patient survives the 24−48 hour critical phase, a gradual reabsorption of extravascular compartment fluid takes place in the following 48−72 hours.

General wellbeing improves, appetite returns, gastrointestinal symptoms abate, hemodynamic status stabilizes, and diuresis ensues. Some may experience generalized pruritus. Bradycardia and electrocardiographic changes are common during this stage.

The hematocrit stabilizes or may be lower due to the dilutional effect of reabsorbed fluid. The white blood cell count usually starts to rise soon after defervescence but the recovery of the platelet count is typically later than that of the white blood cell count

PATHOPHYSIOLOGY

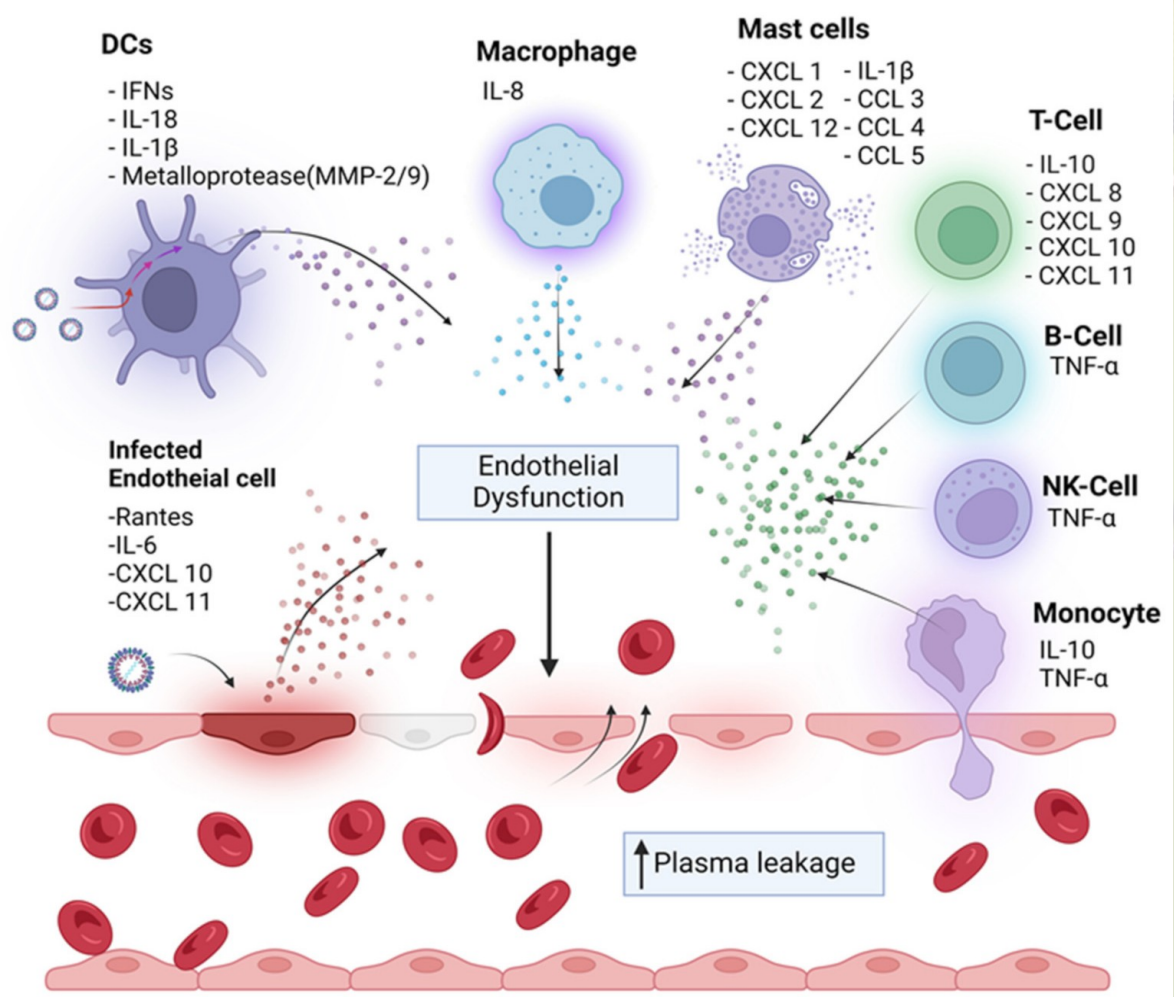
Viral Entry and Replication

- DENV enters the body via the bite of an infected *Aedes* mosquito.
- It targets dendritic cells and monocytes, entering through receptor-mediated endocytosis.
- Once inside, the virus replicates in host cells, producing viral proteins and RNA.
- New virions are released, spreading the infection systemically.

Immune Response and Cytokine Storm

- The innate immune system responds with interferons and pro-inflammatory cytokines.
- In secondary infections, **antibody-dependent enhancement (ADE)** may occur:
  - Non-neutralizing antibodies from a previous infection facilitate viral entry into immune cells.
  - This leads to increased viral load and heightened immune activation.
- A **cytokine storm** can result, contributing to vascular permeability and tis

PATHOPHYSIOLOGY



PLATELETS ACTIVATION

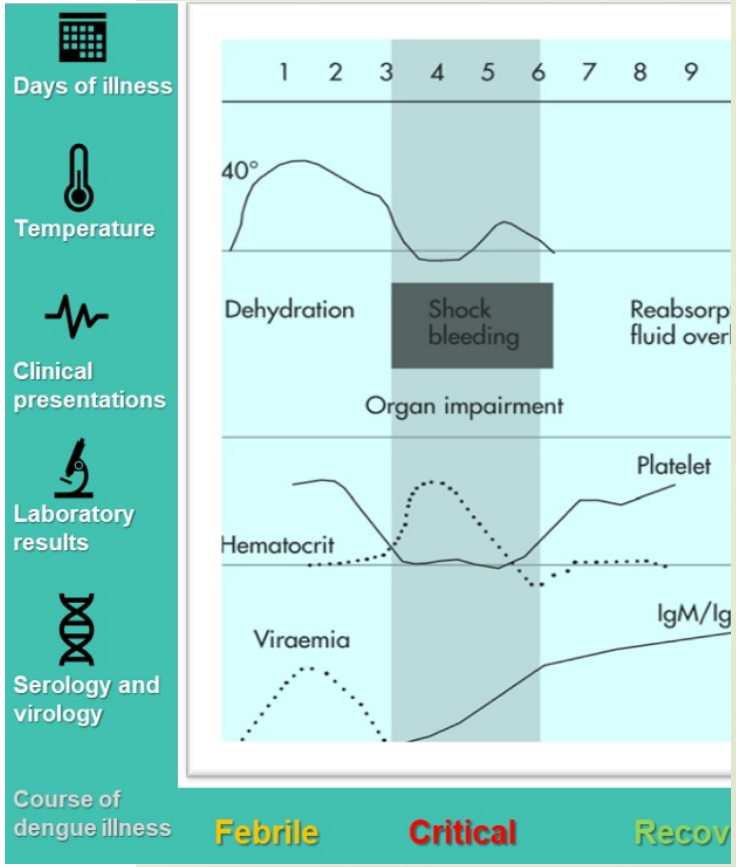
- Platelets from dengue patients present signs of activation, mitochondrial dysfunction and activation of apoptosis caspase cascade resulting in thrombocytopenia. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) also termed as CD209 has been shown as a critical receptor involved in DENV-dependent platelet activation (Hottz et al., 2013b).

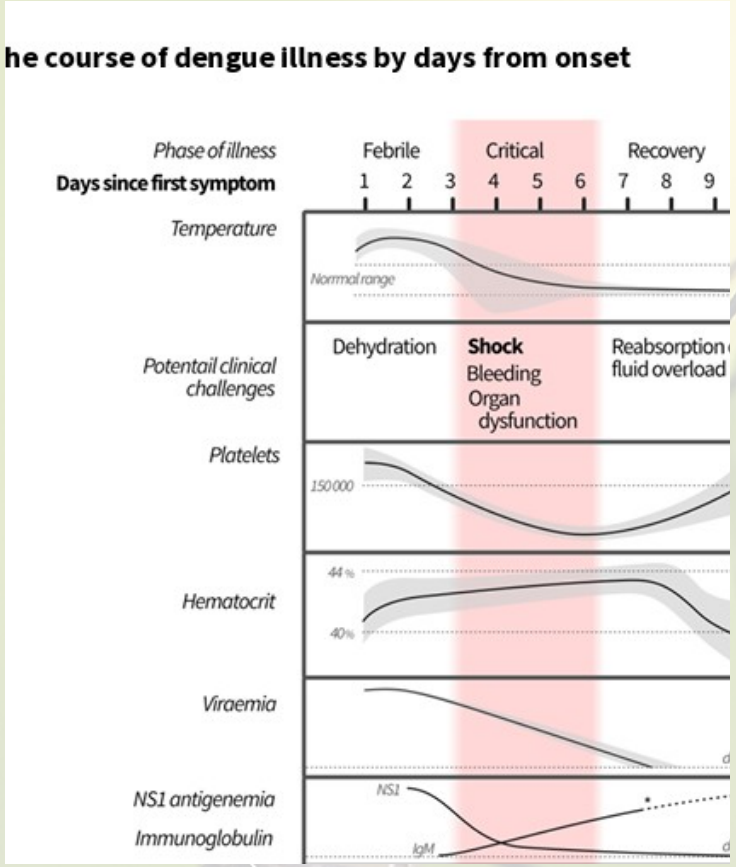
- DENV directly binds to the surface receptors DC-SIGN, heparan sulfate proteoglycan (HSP) and CLEC-2 (C-type-lectin-like receptor 2) on platelets and replicates inside the platelets (Simon et al., 2015; Sung and Hsieh, 2019).

DENGUE COMPLICATIONS

Table 1. Medical complications seen in the febrile, critical and recovery phases of dengue

1	Febrile phase	Dehydration: high fever may cause neurological disturbances and febrile seizures in young children
2	Critical phase	Shock from plasma leakage: severe haemorrhage; organ impairment
3	Recovery phase	Hypervolaemia (only if intravenous fluid therapy has been excessive and/or has extended into this period) and acute pulmonary oedema





CLINICAL COURSE - TIMELINE

DIFFERENT IATING FACTORS

WHO-2025

Table 2-2. Clinical manifestations of dengue, chikungunya and Zika which might differentiate them from each other[1]

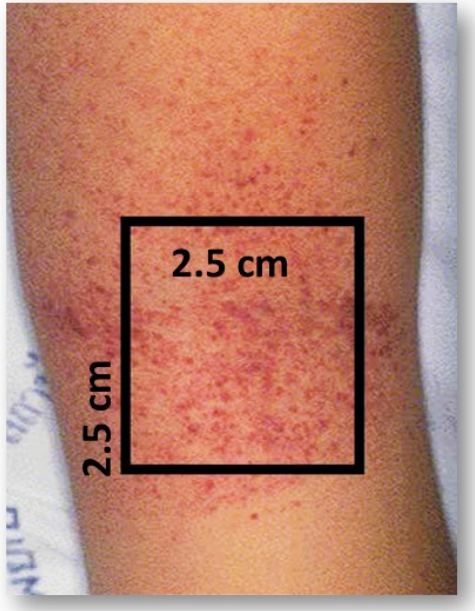
Certainty of the evidence	Manifestations of dengue	Manifestations of chikungunya	Manifestations of Zika
HIGH (findings that differentiate them)	Thrombocytopenia Progressive increase in haematocrit Leukopenia	Arthralgia	Pruritus
MODERATE (findings that probably differentiate them)	Anorexia or Vomiting Abdominal pain Chills Haemorrhage (includes bleeding on the skin, mucous membranes, or both)	Rash Conjunctivitis Arthritis Myalgia or bone pain	Rash Conjunctivitis
LOW (findings that may differentiate them)	Retro-ocular pain Hepatomegaly Headache Diarrhoea Dysgeusia Cough Elevated transaminases Positive tourniquet test	Haemorrhage (includes bleeding on the skin, mucous membranes, or both)	Adenopathy Pharyngitis or odynophagia

[1]Adapted from the Pan American Health Organization guidelines for diagnosis and treatment of dengue, chikungunya, and zika in the Region of the Americas

TORNIQUET TEST

How To Do a Tourniquet Test

1. Take the patient's blood pressure and record it, for example, 100/70.
2. Inflate the cuff to a point midway between SBP and DBP, and maintain for 5 minutes, (100 + 70) ÷ 2 = 85 mm Hg
3. Reduce and wait 2 minutes.
4. Count petechiae below antecubital fossa.
   - A positive test is 10 or more petechiae per 1 square inch.



Dengue Fever

CASE DEFINITION

A. Case Definition:

Case Classifications	Case Definitions
Suspected Case	A person who meets the following clinical picture: - High fever >38.5 with one or more of the following symptoms: • Headache • Joint and muscle pains • Retro-orbital pain • nausea • vomiting • swollen glands • rash
Probable Case	A person who meets the above-suspected case criteria *with* epidemiological links like: 1- Present the entomological index at the case place (e.g., home or work). 2- Live or travel to an endemic case in the last 15 days.
Confirmed Case	A person who meets the above-suspected case criteria with at least one of the following tests: 1- RT-PCR 2- Ns1 3- IgM[1] **Note:** IgG detection by ELISA in a single serum sample is not helpful for diagnostic testing because it remains detectable for life after a dengue virus infection. Except if it has been done to check the secondary infection, two samples must be taken 2-4 weeks part and increased four-folds.
Not a Case	A person who has all[2] the following tests negative/ not detected: 1- RT-PCR 2- Ns1 3- IgM

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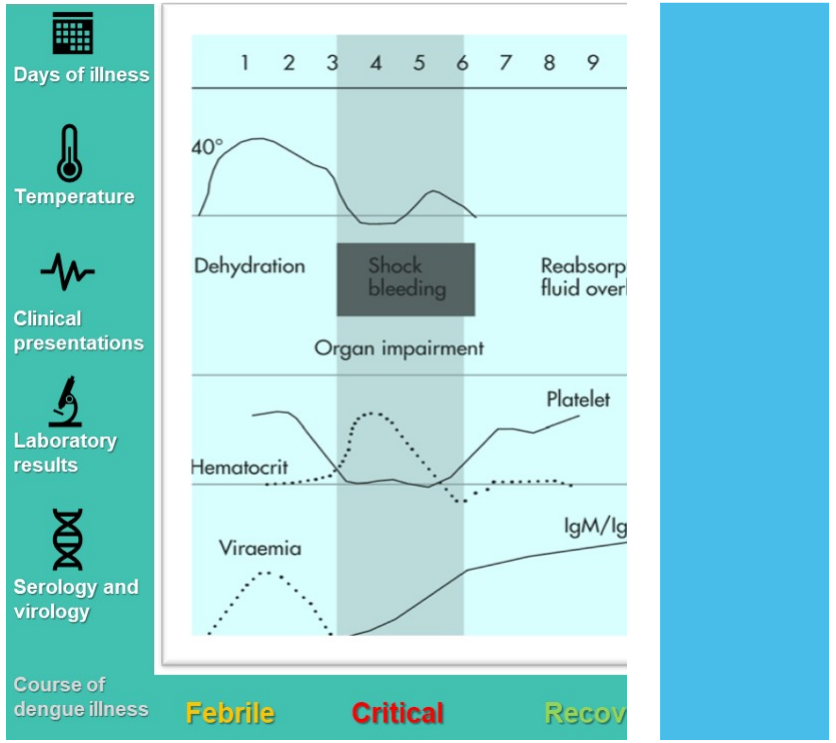
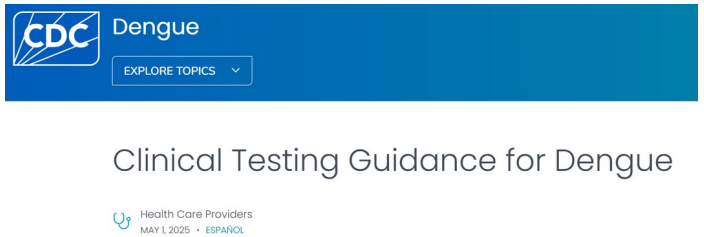
PUBLIC HEALTH AUTHORITY

Dengue Fever Disease:





DIAGNOSIS

Acute phase: initial 0-7 days after symptom onset

During this period, laboratory diagnosis should be made on serum samples using either one of these test combinations:

- A nucleic acid amplification test **(NAAT)** (e.g., RT-PCR) and an **IgM antibody** test **OR**
- A **NS1 ELISA** test and an **IgM antibody test**

A serum sample is preferred for dengue testing.

Dengue virus RNA can be detected with NAAT in blood, serum, and plasma. Most of these tests identify the infecting dengue virus serotype. **A positive NAAT test does not require further confirmatory testing**.

SENSITIVITY AND SPECIFICITY

A negative result from a NAAT or NS1 ELISA test does not rule out infection.

A positive result by NAAT or NS1 ELISA meets the confirmatory laboratory criteria for diagnosis in the National Notifiable Diseases Surveillance System (NNDSS) dengue case definition.

DIAGNOSIS OF DENGUE FEVER

Convalescent Phase: >7 days post symptom onset

When the acute (0-7 days) sample is negative in the recommended test combinations or is not available, a convalescent serum sample can be collected and tested.

IgM ELISA antibody test is recommended as the primary test after day 7 of symptom onset.

Some patients may be positive on NAAT or NS1 ELISA tests after day 7 of illness.

IgM antibodies can be reliably detected by an IgM antibody test for 3 months or longer after infection.

INTERPRETING TEST RESULTS

Patients with a positive NAAT (E.g., RT-PCR) or NS1 ELISA test have a confirmed acute dengue virus infection.

Patients who have IgM antibodies against dengue virus in a single sample are classified as having a presumptive, recent dengue virus infection.

Patients with a change from negative to positive IgM results in paired samples (first sample collected during the first 7 days of illness, and second sample collected after symptoms subside) are classified as recent dengue infections.

IGG TESTING

Serologic testing by IgG in a single specimen is not recommended for diagnosis of acute dengue in patients, as these tests may detect antibodies from dengue infections or other flavivirus infections that occurred in the past

Patients with a change from negative to positive IgG results in paired samples (first sample collected during the first 7 days of illness, and second sample collected after symptoms subside) are classified as recent dengue infections.

SUMMARY OF DIAGNOSTICS

For symptomatic persons with dengue virus infection, dengue virus RNA can usually be detected by molecular tests for the first 0-7 days in the course of illness. After day 7, molecular tests may not be optimal.

NS1 is detectable during the acute phase of dengue virus infections. NS1 ELISA tests can be as sensitive as molecular tests during the first 0-7 days of symptoms. After day 7, NS1 tests may not be optimal.

IGM & CROSS REACTIVITY

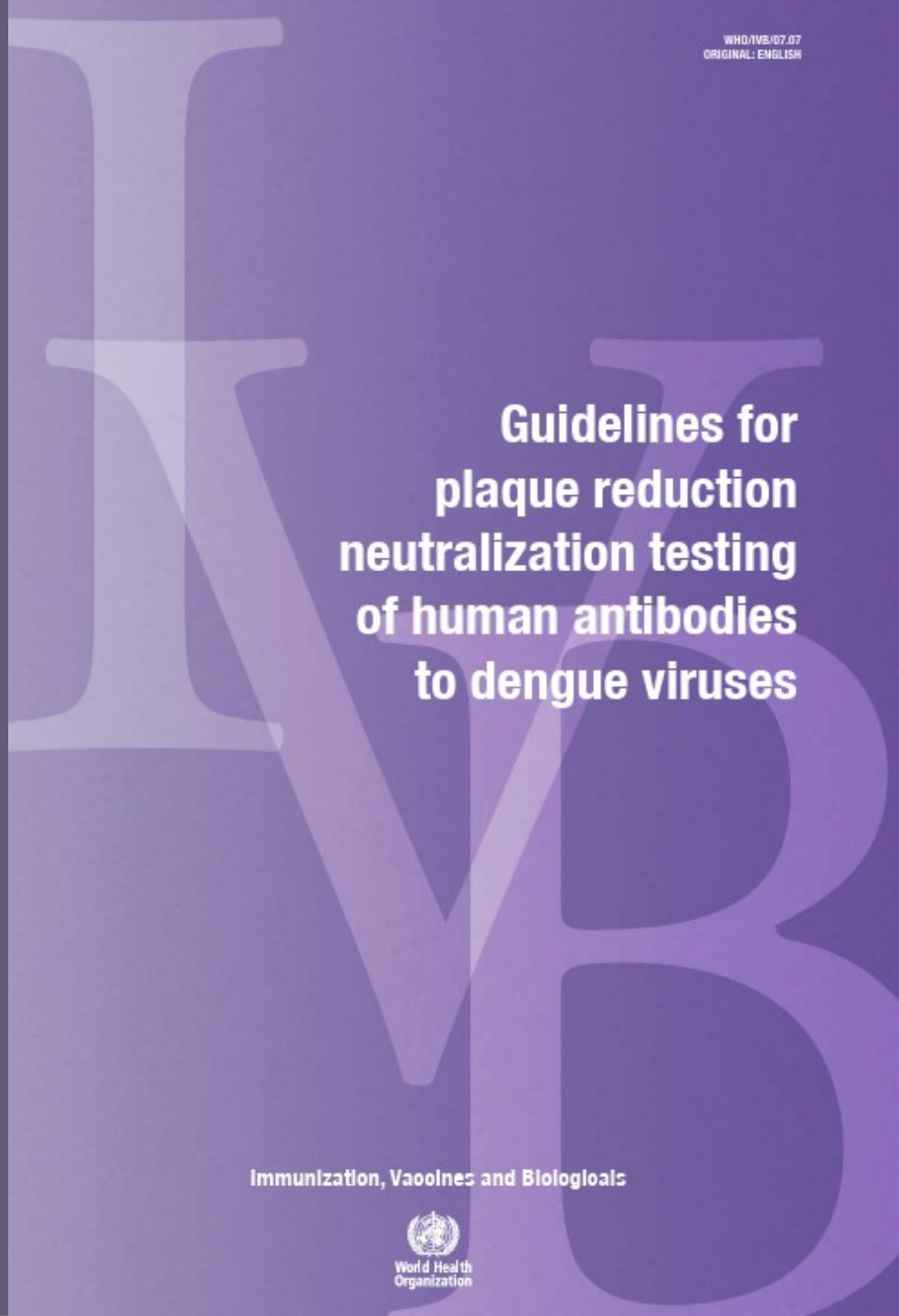
IgM antibody testing can identify most recent dengue infections after day 3 of illness. These tests should be run on samples with negative NS1 ELISA and PCR results, particularly after day 3 of illness. Interpreting positive IgM results is complicated because of cross-reactivity with other flaviviruses, like Zika.

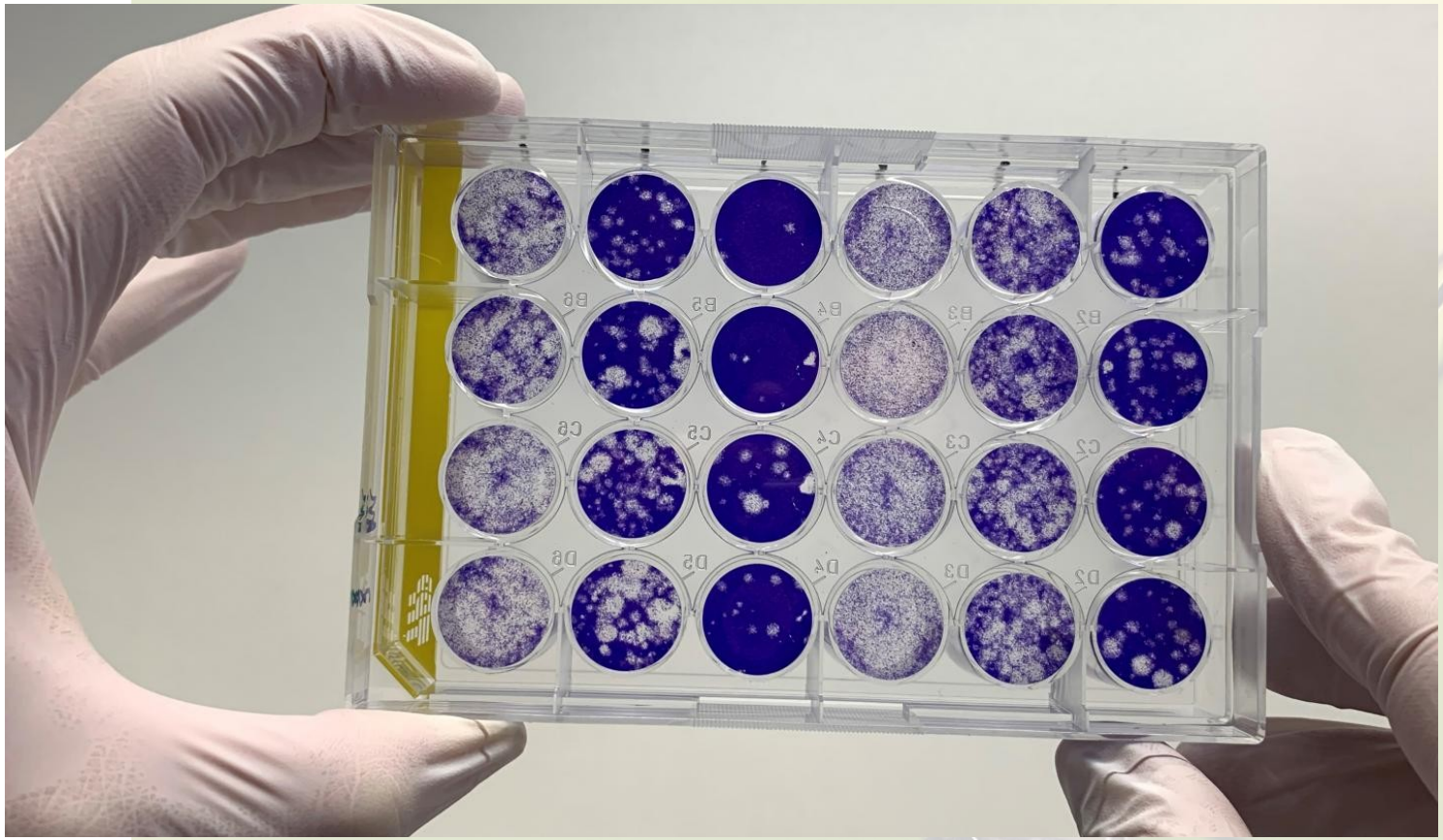
Plaque Reduction Neutralization Tests (PRNT) can resolve false-positive IgM antibody results caused by non-specific reactivity, and, in some cases, can help identify the infecting virus. However, in areas with high prevalence of dengue and Zika virus neutralizing antibodies, PRNT may not confirm a significant proportion of IgM positive results.

PLAQUE REDUCTION NEUTRALIZATION TESTS (PRNT)

- **How PRNT Works**

1. **Serum Preparation**
    1. Patient serum is serially diluted.
    2. Mixed with a known quantity of virus.
2. **Virus-Antibody Incubation**
    1. The mixture is incubated to allow antibodies to bind and neutralize the virus.
3. **Cell Infection**
    1. The mixture is added to a monolayer of susceptible cells.
    2. A semi-solid overlay (e.g., agar or methylcellulose) is added to restrict viral spread.


WHO/IVB/07.07
ORIGINAL: ENGLISH
Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses
Immunization, Vaccines and Biologicals
World Health Organization



PLAQUE REDUCTION NEUTRALIZATION TESTS (PRNT)

Plaque Formation

- After incubation, plaques (areas of cell destruction) form where virus infects cells.
- Fewer plaques = more neutralizing antibodies.

Titer Calculation

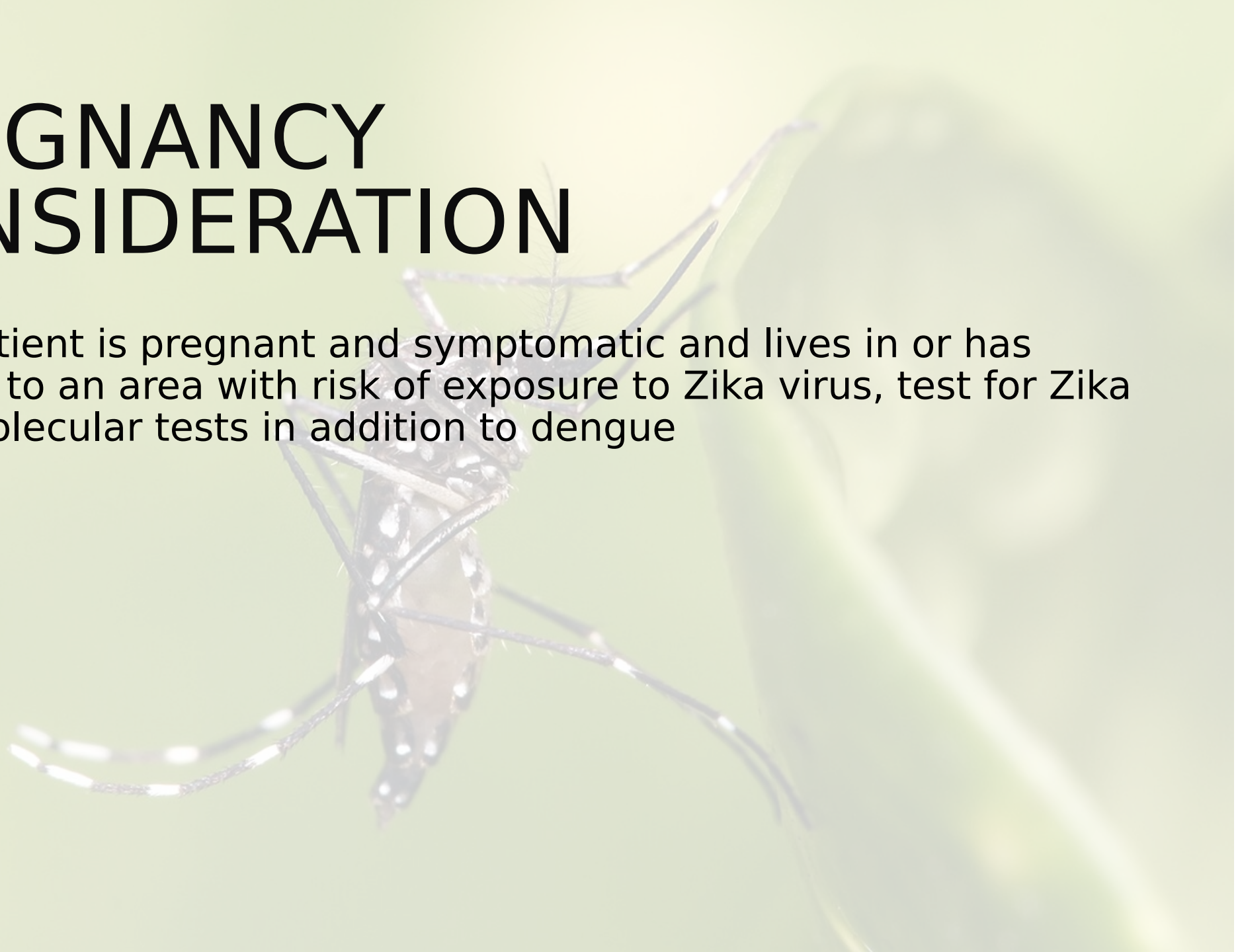
- The dilution that reduces plaque count by 50% is called **$PRNT_{50}$**.
- $PRNT_{90}$ is also used for higher stringency.

CROSS REACTIVITY

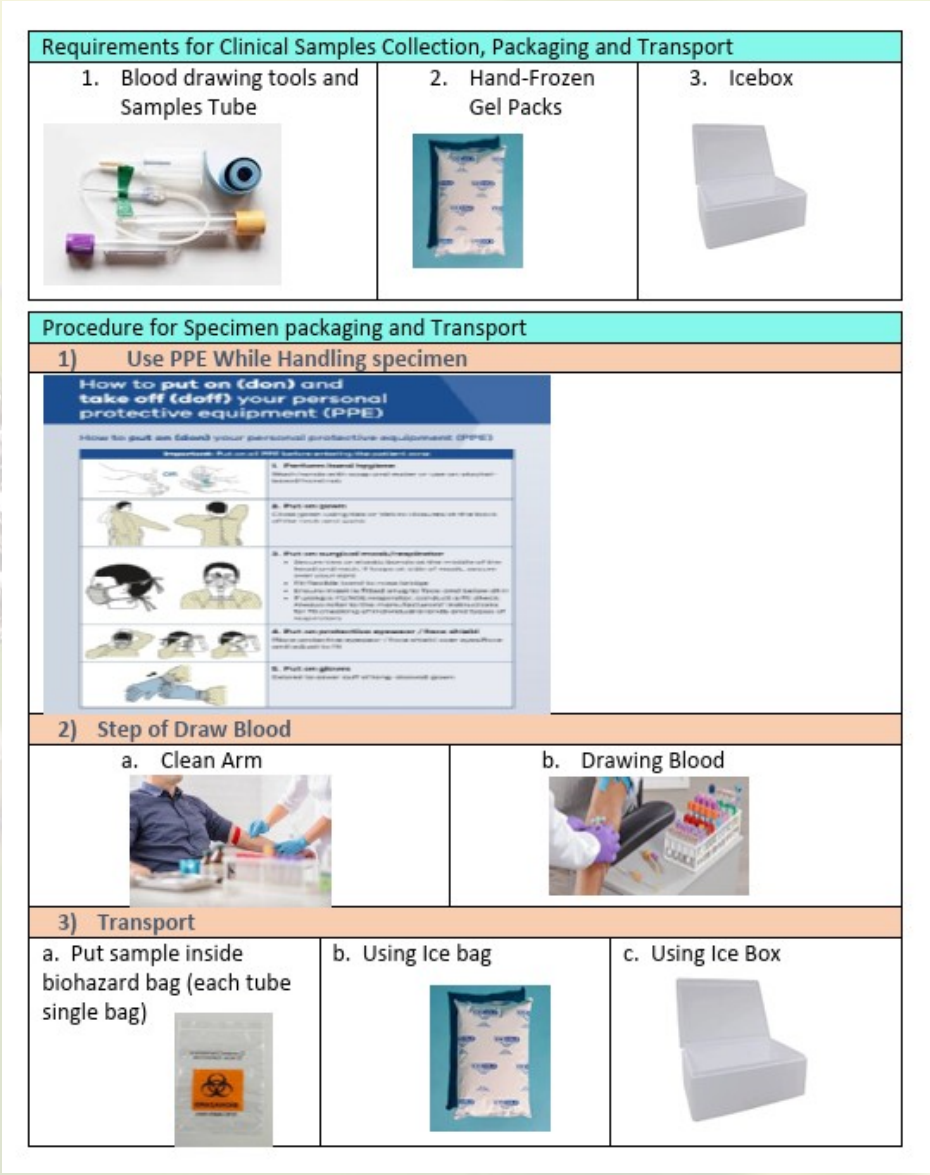
Cross reactivity is a limitation of dengue serological tests and is seen when antibodies against other flaviviruses react on the dengue IgM ELISA test. For people living in or traveling to an area with concurrently circulating flaviviruses, clinicians will need to order plaque reduction neutralization test (PRNT) to rule out dengue on IgM-positive specimens. Zika, Japanese encephalitis, St. Louis encephalitis, West Nile, and yellow fever viruses are examples of other flaviviruses to be considered when ruling out dengue by PRNT. PRNT does not always a give conclusive diagnostic result, particularly in patients that have previously been exposed to more than one flavivirus. Current dengue molecular tests (E.g., RT-PCR) and NS1 ELISA tests do not have cross-reactivity with other flaviviruses of concern.

PREGNANCY CONSIDERATION

If the patient is pregnant and symptomatic and lives in or has traveled to an area with risk of exposure to Zika virus, test for Zika using molecular tests in addition to dengue



1. Two Separate serum/plasma samples within (2) hours of blood collection.
2. Refrigerate serum specimens (two samples) at a temperature of 2-8°C.
3. And during shipping specimens (two samples) to the laboratory, stored them at a temperature of 4°C on ice packs within 24 hours.
4. Any anticipated delay of more than 48 hours before testing must store them at a freezing temperature of -20°C.
5. And then ship the specimens (two samples) on dry ice if frozen.
6. Avoid any repeated freezing and thawing.


Requirements for Clinical Samples Collection, Packaging and Transport
1. Blood drawing tools and Samples Tube
2. Hand-Frozen Gel Packs
3. Icebox
Procedure for Specimen packaging and Transport
1) Use PPE While Handling specimen
How to put on (don) and take off (doff) your personal protective equipment (PPE)
2) Step of Draw Blood
a. Clean Arm
b. Drawing Blood
3) Transport
a. Put sample inside biohazard bag (each tube single bag)
b. Using Ice bag
c. Using Ice Box

SCREENING FOR OTHER VHF

If all dengue tests are negative, **screen 25% of “not-a-case” samples** for other VHFs:

Alkhurma Hemorrhagic Fever Virus (AHFV)

Rift Valley Fever Virus

Yellow Fever Virus

West Nile Virus

Zika Virus

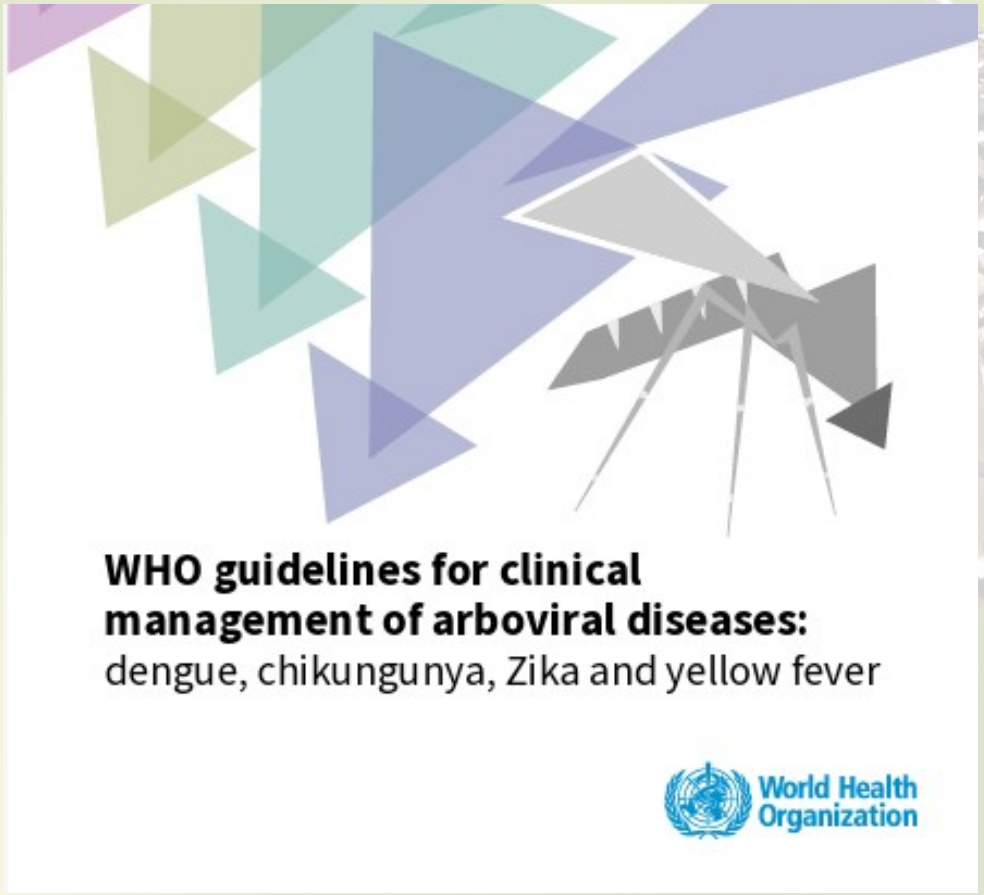
Chikungunya Virus

WHO GUIDELINES 2025

Recommendations specific to patients with severe (hospitalized), suspected or confirmed arboviral disease (dengue, chikungunya, Zika and yellow fever):

- WHO suggests using crystalloid fluid rather than colloid fluid in patients who require intravenous fluid treatment for suspected or confirmed severe arboviral disease. [Conditional recommendation, low certainty evidence]
- WHO recommends the use of capillary refill time to guide intravenous fluid management in patients with suspected or confirmed severe arboviral disease. [Strong recommendation, low certainty evidence]
- WHO recommends the use of lactate measurement in addition to standard care to guide intravenous fluid management in patients with suspected or confirmed severe arboviral disease. [Strong recommendation, moderate certainty evidence]
- WHO suggests the use of passive leg raise test in patients in shock, with suspected or confirmed arboviral disease, when the clinician is uncertain if further intravenous fluid administration is warranted. [Conditional recommendation, low certainty evidence]
- WHO suggests against the use of systemic corticosteroids in the treatment of patients with suspected or confirmed severe arboviral disease. [Conditional recommendation, very low certainty evidence]
- WHO suggests against the use of immunoglobulins in the treatment of patients with suspected or confirmed severe arboviral disease. [Conditional recommendation, very low certainty evidence]
- WHO suggests against the use of prophylactic platelet transfusion in patients with suspected or confirmed severe arboviral disease and platelet count of <50,000 platelets/microlitre who have no active bleeding. [Conditional recommendation, low certainty evidence]
- WHO suggests the use of intravenous N-acetylcysteine in the treatment of patients with liver failure due to suspected or confirmed yellow fever. [Conditional recommendation, very low-certainty evidence]
- WHO recommends the use of monoclonal immunoglobulin TY014 in the treatment of patients with yellow fever only in research settings. [Use only in research]
- WHO recommends the use of sofosbuvir in the treatment of patients with yellow fever only in research settings. [Use only in research]

WHO GUIDELINES 2025


WHO guidelines for clinical management of arboviral diseases:
dengue, chikungunya, Zika and yellow fever
World Health Organization

Recommendations for patients with non-severe, suspected or confirmed arboviral diseases (dengue, chikungunya, Zika and yellow fever):

- WHO suggests the use of protocolized oral fluid treatment compared with non-protocolized oral fluid treatment in patients with suspected or confirmed non-severe arboviral disease. [Conditional recommendation, low certainty evidence]
- WHO suggests the use of paracetamol for the treatment of pain and/or fever in patien with suspected or confirmed non-severe arboviral disease. [Conditional recommendation, low certainty evidence]
- WHO suggests the use of metamizole for the treatment of pain and/or fever in patient with suspected or confirmed non-severe arboviral disease. [Conditional recommendation, low certainty evidence]
- WHO recommends against the use of non-steroidal anti-inflammatory medications (NSAIDs) in patients with acute suspected or confirmed arboviral disease, irrespectiv of severity. [Strong recommendation, low certainty evidence]

DENGUE IN SAUDI ARABIA

Dengue was first reported in KSA in 1994 in Jeddah.

Dengue virus serotype 2 (DENV-2) was identified in that patient , and was responsible for a significant epidemic in Jeddah, KSA, with 289 confirmed cases reported. Since then, dengue cases have been reported in other geographical locations of KSA

DENGUE IN SAUDI ARABIA

6637 subjects were screened for DENG fever in the southwest region of Jazan in Saudi Arabia.

This study data was collected over 9years i.e., 2012–2020 in17different hospitals in Jazan city. In 2012, 2.3%(n=153) patients were diagnosed for DENG fever. In 2013 (3.1%, n=206), 2014 (2.3%, n=153), 2015 (7.1%, n=469), 2016 (3.0%, n=197), 2017 (8.5%, n=567), 2018 (7.1%, n=470), 2019 (42.7%,n=2834) and 2020 (23.9%,n=1588).The maximum number of enrolled cases were documented in 2019(42.7%) and then 2020 (23.9%)

Between 2012 and 2014, 2.3% were the minimum cases were enrolled.

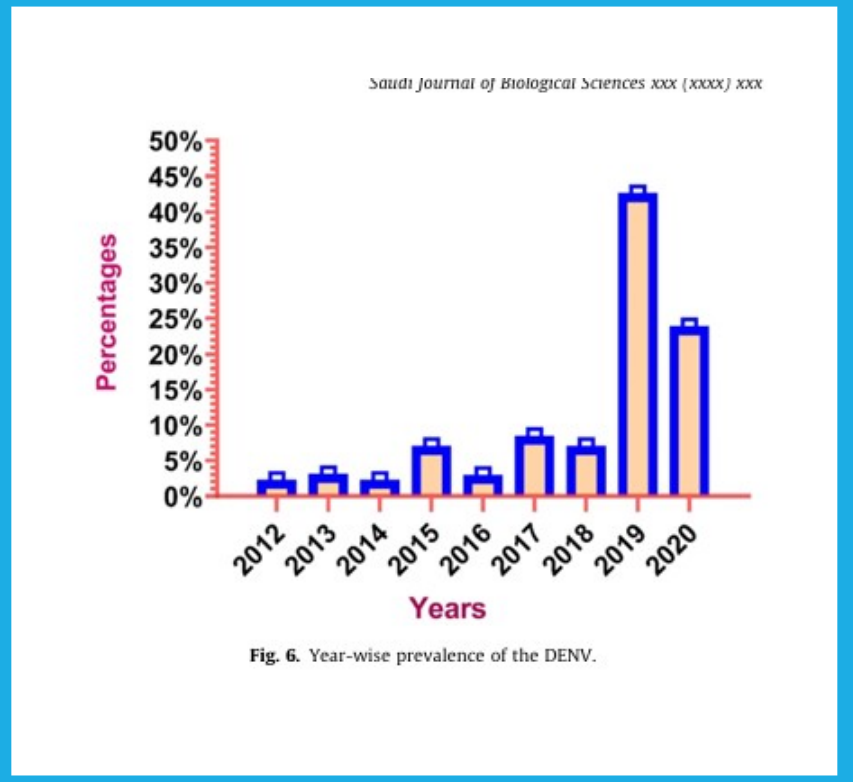


Fig. 6. Year-wise prevalence of the DENV.

Saudi Journal of Biological Sciences xxx (xxxx) xxx
Contents lists available at ScienceDirect
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journal homepage: www.sciencedirect.com
Original article
Epidemiological screening and serotyping analysis of dengue fever in the Southwestern region of Saudi Arabia
Alkhansa Alshabi, Amani Marwan, Nuzhath Fatima, Aymen M. Madkhali, Fatemah Alnagai, Abrar Alhazmi, Hesham M. Al-Mekhlafi, Ahmed A. Abdulhaq, Khalid Y. Ghailan, Ahmed Sali, Tareq Refaei

DENGUE IN SAUDI ARABIA

ELSEVIER

Journal of Infection and Public Health

Volume 9, Issue 2, March–April 2016, Pages 117-124

Review

The epidemiology of Dengue fever in Saudi Arabia: A systematic review

Alaa Alhaeli, Salwa Bahkali, Anna Ali, Mowafa S. Househ, Ashraf A. El-Metwally

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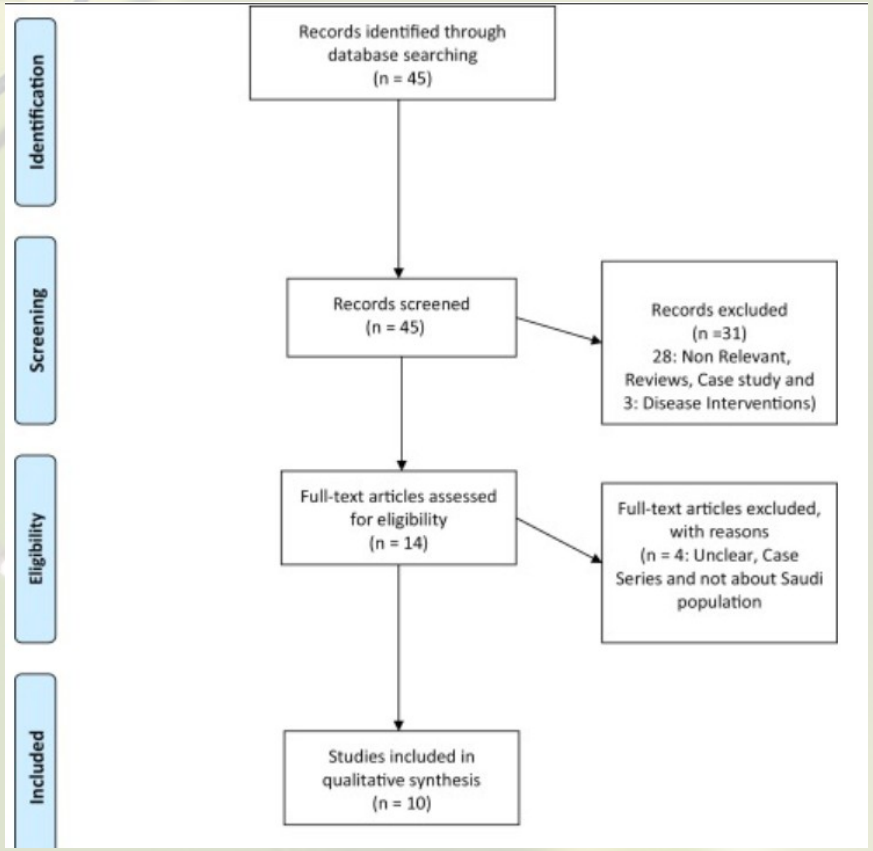
Identification
Screening
Eligibility
Included
Records identified through database searching (n = 45)
Records screened (n = 45)
Records excluded (n =31) 28: Non Relevant, Reviews, Case study and 3: Disease Interventions)
Full-text articles assessed for eligibility (n = 14)
Full-text articles excluded, with reasons (n = 4: Unclear, Case Series and not about Saudi population
Studies included in qualitative synthesis (n = 10)

RISK FACTORS

- A study by Khormi et al. in 2012 revealed the following risk factors: mosquito counts, population density in inhabited areas, total population per district, water access, neighborhood quality. Increased mean population density was a significant factor in determining the risk for DF
- A case–control study conducted in 2012 reported the following significant risk factors for DF: presence of stagnant water in indoor drainage holes, indoor larvae, nearby construction sites, and old age

المجلة الصحية لشرق المتوسط — المجلد الثامن عشر العدد الأول

Factors associated with the spread of dengue fever in Jeddah Governorate, Saudi Arabia

A.A.N. Kholedi,[1] O. Balubaid,[2] W. Milaat,[3] I.A. Kabbash[4] and A. Ibrahim[5]

العوامل المصاحبة لانتشار حمى الضنك في محافظة جدة، المملكة العربية السعودية

أحمد عبد الله ناصر الخليدي، عمر باليد، وليد ملعاط، إبراهيم علي كباش، عادل إبراهيم

الخلاصة: انبعثَتْ حمى الضنك من مرقدها انبعاثاً عنيفة في جدة عام 2004 ليصل عدد الحالات عام 2006 إلى 1308 حالة. وتسعَى هذه الدراسة المشهَّدة إلى تحديد العوامل التي تُعزِّز انتشار الداء، تمهيداً لتحديد الخط القاعدي من القيم الوبائية والذي يساعد على مكافحة حمى الضنك. وقد كانت جميع الحالات المشتبه بإصابتها (وعددها 650 حالة) من حمى الضنك في جدة عام 2007 مؤهلة للاندراج ضمن الدراسة. وتمثَّلت الحالات المرضية في تلك الحالات التي تأكدت إصابتها بحمى الضنك بالدراسات المختبرية (وعددها 244)، في حين تمثَّلت الشواهد في الحالات التي تأكدت سلبيتها (وعددها 406). ومن بين المجموع كان هناك 129 حالة دراسة و240 شاهد أمكن للباحثين التواصل معها وأدرجت ضمن الدراسة. وقد أدرج الباحثون العوامل التي يُعتدُّ بها إحصائياً في التحليل الثنائي المتغيرات ضمن التحليل التحوُّفي اللوجستي. وتبيَّن لهم أن المحددات المستقلة للعدوى بحمى الضن (P>0.01) تتمثَّل في وجود مياه راكدة في فجوات نزح المياه داخل المباني (OR = 4.9)، ووجود اليرقات داخل المباني (OR = 2.2). ووجود مواقع للبناء قريبة (OR = 2.2)، والتقدم في العمر (OR = 1.2) كما تبيَّن للدارسين أن التثقيف الصحي بالتواصل المباشر قد أنقص من خطر العدوى بحمى الضنك إنقاصاً يُعتدُّ به إحصائياً. وأن الحاجة ماسّة لبذل الجهود لمكافحة العوامل التي تم تحديدها في هذه الدراسة والتي هي ممكنة التعديل، مع التأكيد على التثقيف الصحي.

ABSTRACT Dengue fever resurged sharply in Jeddah in 2004 and rose to 1308 cases in 2006. This case–control study determined factors potentiating the spread of the disease to provide an epidemiological baseline to help dengue control. All (650) suspected cases of dengue in Jeddah in 2007 were eligible for inclusion. Cases were those confirmed with dengue by laboratory investigations (n = 244) and controls those confirmed negative (n = 406). Of these, 129 cases and 240 controls could be contacted and were included in the study. Variables found significant in the bivariate analysis were included in a logistic regression analysis. The presence of stagnant water in indoor drainage holes (OR = 4.9), indoor larvae (OR = 2.2), nearby construction sites (OR = 2.2), and older age (OR = 1.2) were independent determinants of dengue infection (P < 0.01 for all). Face-to-face health education significantly decreased the risk of dengue infection. Efforts are needed to control the modifiable factors identified in this study with emphasis on health education.

1 May 2012
Vol. 6 No. 2 (2012) | https://doi.org/10.4081/gh.2012.135

Assessing the risk for dengue fever based on socioeconomic and environmental variables in a geographical information system environment

Hassan M. Khormi

Table 1. Studies conducted in different region of Saudi Arab related to DF.

Author, year, reference	Regions/cities	Sample size	Study design	Main findings
Prevalence studies and risk factors studies				
Memish et al. (2011) [14]	Jazan	Serological evaluation of 1,026 soldiers	Cross-sectional	Low sero-prevalence of DF[1] (0.1%).
Al-Azraqi et al. (2013) [15]	Jizan and Aseer cities	965 random sample of patients attending the outpatients' clinics of for	Cross-sectional	31.7% positive cases. Risk factors were male gender and younger age (15–29 years), lack of electricity and having water basins in the house.
Ahmed MM (2010) [21]	Jeddah	147 patients infected with dengue	Cross-sectional	Clinical presentations=fever, vomiting and abdominal pain. Hematological abnormalities=thrombocytopenia, leucopoenia and a WBC of <4.0×109/L.
Alzahrani et al. (2013) [19]	Jeddah	2288 dengue cases	Cross-sectional	Risk factors were male gender and younger age 15–29 years.
Laboratory confirmed cases and clinical findings				
Ayyub et al. (2006) [20]	Jeddah	80 patients with a suspected diagnosis of DF and Dengue Hemorrhagic Fever	Cross-sectional	Male to female ratio 3.3:1 Mean age at infection=27years. summer months symptoms=fever, headache, myalgia and vomiting. Main hematological abnormalities=thrombocytopenia and leucopenia.
Khan et al. (2008) [7]	Makkah	160 clinically suspected patients	Cross-sectional	91=laboratory confirmed dengue. Young adults, median age=26 years, male:female ratio=1.5:1. Symptoms=fever, malaise,

Distribution of mosquitoes and the first record of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae) in the Riyadh Province, Kingdom of Saudi Arabia

Mohammed H Alzahrani, Yousif Eldirdiry Elamin, Mohammed Abdullah Al Helal, Abdelmohsin MO Abdoon, Waleed M EL-Motasim Ibrahim, Faris ahmed Alqarni, Hasabelrasol FA Elhaj and Mo'awia Mukhtar Hassan

Abstract

Mosquitoes are among the most important vectors that transmit several parasitic and viral diseases in different regions of the world. Mosquito surveillance was carried out from 2012 to 2017 to determine the occurrence and distribution of mosquito species in five places in the Riyadh Province (Riyadh city, Al-Kharj, Ad Dilam, Al Majma'ah, and Al Duwadimi) in the Kingdom of Saudi Arabia (KSA). Larval collections were carried out using standard dippers and adult collections with Bio traps. The mosquito collection comprised *Anopheles dthali*, *An. pretoriensis*, *Culex pipiens*, *Cx. quinquefasciatus*, and *Aedes aegypti*. The presence of *Ae aegypti* is considered to be the first record in the mentioned places. The occurrence of *Ae. aegypti* represents a serious risk of transmission of *Ae. aegypti*-borne diseases in the region. Therefore, active entomological surveillance and proper vector control are needed to prevent the further spread of this vector and the transmission of *Ae. aegypti*-borne diseases in the region.

Table 3: Number of *Aedes aegypti* collected in different surveyed areas in the Riyadh Province from 2012 to 2017.

Governorate/ City	Year	Number of *Aedes aegypti*		Total
		Larvae	Adults	
Riyadh city	2012	0	0	0
Al-Kharj Governorate		0	4	4
Al Majma'ah Governorate		0	10	10
Al Duwadimi Governorate		0	4	4
Riyadh city	2013	0	0	0
Al-Kharj Governorate		0	25	25
Al Majma'ah Governorate		0	37	37
Al Duwadimi Governorate		0	18	18
Riyadh city	2104	0	4	4
Al-Kharj Governorate		0	0	0
Al Majma'ah Governorate		0	10	10
Al Duwadimi Governorate		0	0	0
Riyadh city	2015	0	1	1
sAl-Kharj Governorate		0	1	1
Al Majma'ah Governorate		0	0	0
Al Duwadimi Governorate		0	0	0
Riyadh city	2016	0	0	0
Al-Kharj Governorate		0	3	3
Al Majma'ah Governorate		0	3	3
Al Duwadimi Governorate		0	0	0
Riyadh city	2017	53	13	66
Al-Kharj Governorate		12	0	12
Al Majma'ah Governorate		0	51	51
Al Duwadimi Governorate		0	0	0
Total		65	184	249

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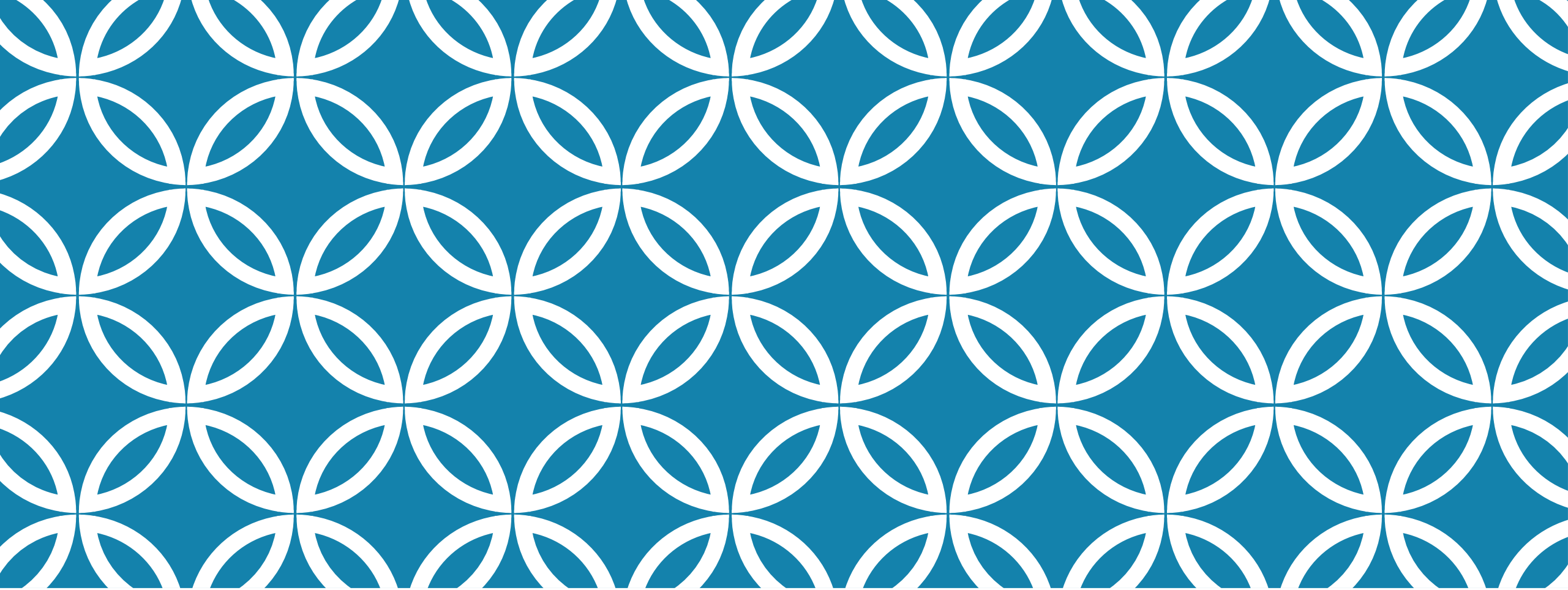

THANK YOU FOR YOUR ATTENTION

Any questions?